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Department of Parasitology, Polish Academy of Sciences
Head: Prof. Dr. Witold Stefański, Member of. P. A. S.

Jadwiga KOZICKA

Metacercaria *Neodiplostomum pseudattenuatum* (Dubois, 1928) Dubois, 1932, syn. *Neodiplostomulum scardinii* Schulman (in Dubinin 1952).

While studying the fish in several of the Mazury Lakes as part of a collective research project on the circulation of parasites in the biocenosis of lakes, headed by the late Professor W. L. Wiśniewski, the author discovered in the brain of fish belonging to the *Cyprinidae* family, mainly in *Scardinius erythrophthalmus* (L.) the metacercaria of *Neodiplostomum pseudattenuatum* (Dubois 1928) Dubois 1932. The metacercaria has not been reported in Poland previously. It was originally described by Schulman as *Neodiplostomulum scardinii* and has been reported so far only in the Soviet Union. Schulman's original description was not available to the author. A short description of metacercaria was given by Dubinin (1952) who announced the appearance of Schulman's paper.

In 1958 Sudarikov identified *Neodiplostomulum scardinii* Schulman as the metacercaria of *Neodiplostomum pseudattenuatum*. A description of the metacercaria of *Neodiplostomum pseudattenuatum* is included herein. Its pathogenicity in regard to fish and deleterious effect on the abundance of fry was described elsewhere, (Kozicka 1958), where the metacercaria was referred to as *Neascus scardinii* (Schulman). The definitive form of the parasite was unknown at that time.

Metacercaria and its development

Metacercariae of *Neodiplostomum pseudattenuatum* settle in the brain cells and ependymal brain layer of fish and fry of *Cyprinidae*, mostly in *Scardinius erythrophthalmus* (L.) in which were found to occur also in the eyes. They are white, mobile and encyst not before

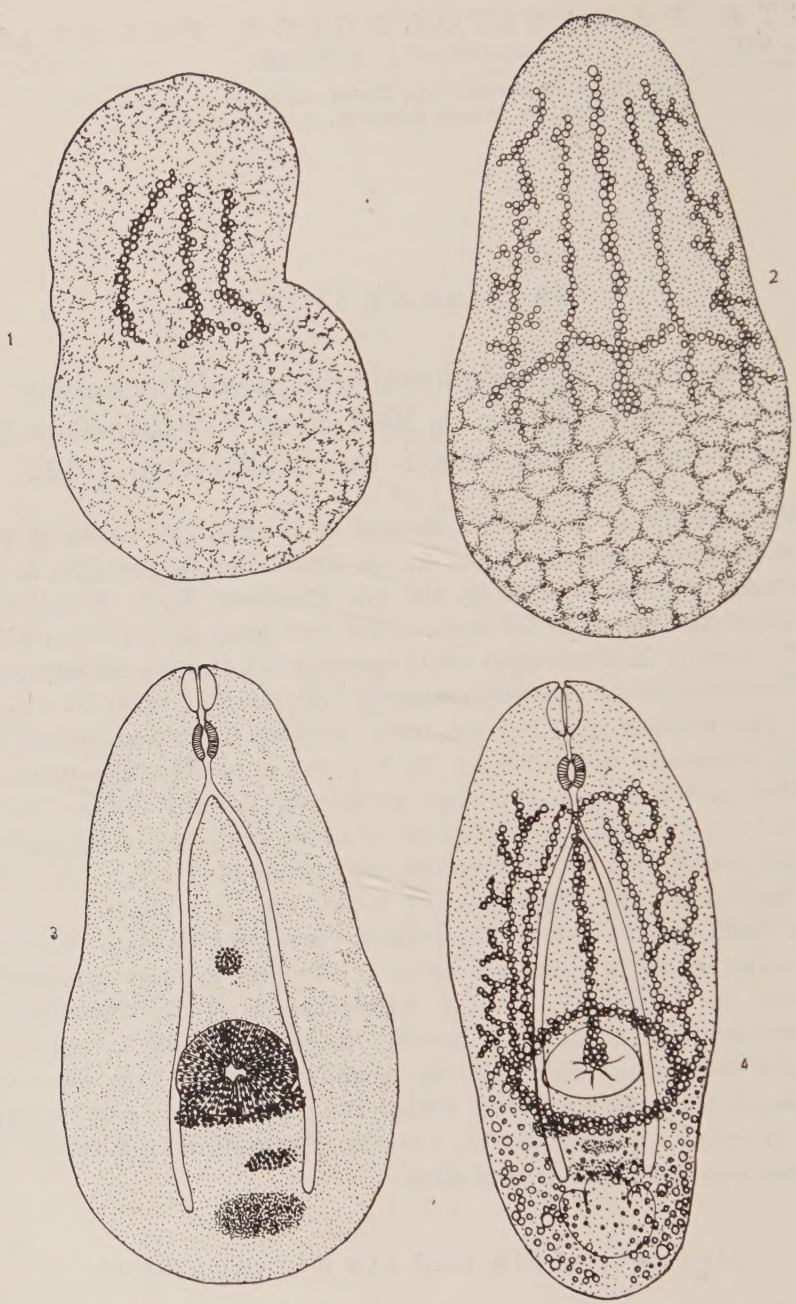

Fig. 1—4. Metacercarie of *N. pseudattenuatum* (D u b.). 1 — young form with three fragments of canals of the „reserve bladder", intravital, 2 — a form a little older as in fig. 1, with 5 canals, infravital. 3 — the older form, from preparation, 4 — the nearly definitive form, infravital.

some time after penetration. Older forms are easily observable with naked eye. The youngest form that could be differentiated was about 0.352 mm long by 0.308 mm wide, oval, opaque and resembling in color the brain tissue. No details of internal morphology could be observed. The cuticle is thin and contours delicate. Metacercariae lie freely in the ependymal tissue. Anterior part of the body is narrower than posterior.

With further development the difference between the two parts of the body become more pronounced (Fig. 1). In the anterior, flatter, leaf-like and slightly narrower part appear fragments of longitudinal canals, referred to as „reserve bladder" marked by long rows of excretory granules. The posterior part is thicker and wider than the anterior one. Both, similarly as in younger forms are opaque. With increasing number of excretory granules the larva becomes surrounded by a slight and delicate, initially scarcely apparent coat.

The subsequent stage (Fig. 2) brings about together with growth a further increase of morphological and structural differences between both parts of the body. The anterior part becomes more narrow yet in comparison to the posterior one and attenuated anteriorly. The posterior part, still thicker and wider becomes rounded at the posterior extremity. The anterior part contains five long excretory canals connecting with one another posteriorly by means of commissures. The median canal has a typical widening at the posterior end, while at the lateral one appear the primordia of the lateral net in the form of branches. The posterior part is filled with light vesicles surounded by minute dark granules thus resembling a net with large loops. The specimens mounted in toto in Canada balsam measure from 0.589 to 0.760 mm by 0.323 to 0.437 mm.

On stained preparation of a slightly older form (Fig. 3) the digestive tract becomes observable: oral sucker, praepharynx, pharynx, a moderately long esophagus and sometimes also the caeca. The ventral sucker is weaker than the oral one and slightly smaller, lying at a fairly good distance from the holdfast organ, slightly above the centre of the body. The holdfast organ is large, slightly flattened at the posterior side. A narrow, multilayer band of glandular cells adheres closely to its posterior margin. Posteriorly to it are situated the primordia of reproductive organs and slightly below the primordium of future copulatory apparatus. In live specimens

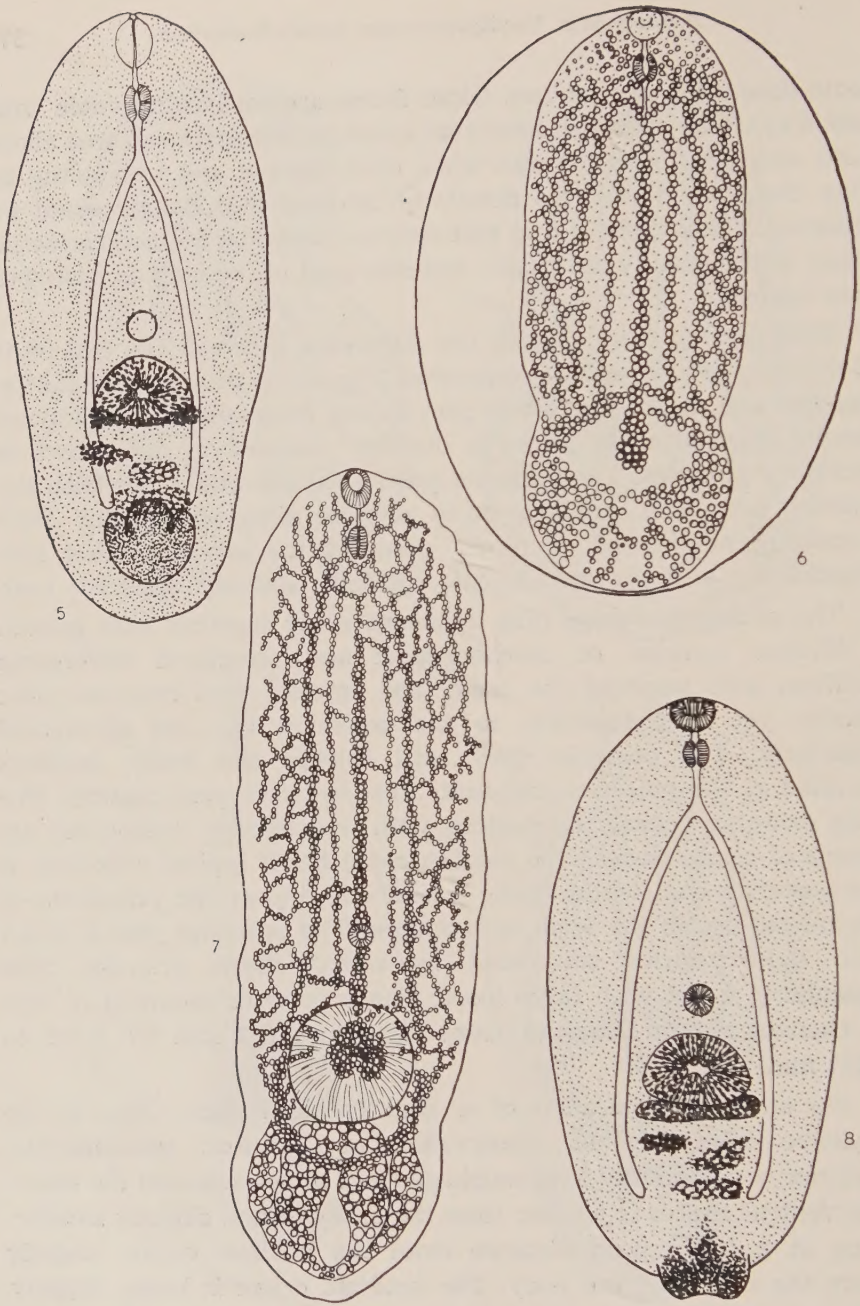

Fig. 5—8. Metacercariae of *N. pseudattenuatum* (D u b.). 5 — the nearly definitive form, from preparation, 6 — the definitive form in the cyst, infravital, 7 — the definitive form liberated from the cyst, infravital, 8 — the definitive form after preparation.

a circular excretory canal is observable. Cuticula is still thin. The specimens stain easily. Cyst walls are still delicate and faintly outlined.

Measurements of specimens mounted in Canada balsam:

body	0.532—0.798 mm by 0.342 by 0.494 mm
oral sucker	0.036 by 0.028; 0.040 by 0.032 mm
pharynx (length)	0.024; 0.028 mm
ventral sucker (diameter)	0.024; 0.028 mm
holdfast organ	0.068—0.092 by 0.100—0.120 mm
primordia of gonads	0.052 by 0.028; 0.040 by 0.040; 0.040 by 0.060 mm
primordium of copulatory apparatus	0.072—0.080 by 0.100—0.120 mm

During subsequent stages growth of the anterior segment relatively to the posterior one takes place. The posterior part becomes much narrower in relation to the anterior one. The body measurements decrease slightly in comparison to the preceding form. Further details of reserve bladder structure become apparent. Thickening of loops of the marginal net and differentiation of primordia of reproductive organs are taking place. The nearly completely (Fig. 4 and 5) and definitively (Fig. 6, 7, 8) formed larval body consists of two parts faintly separated from one another. The anterior part seen from the side is wedge-like, sometimes curved to the ventral side and widening gradually toward the posterior. It joins the posterior part which is slightly wider from it and, therefore, thicker. The general appearance of the body seen either from the ventral or dorsal side may be oval without narrowing (Fig. 8) or narrowed as a result of contraction at the level of the upper or lower margin of the holdfast organ (Fig. 6, 7). The anterior part, flat and leaf shaped, slightly depressed dorso-ventrally is at this stage much longer from the posterior one and may be either slightly thicker or slightly widen (Fig. 4) or possibly equally wide to it (Fig. 6). These relations are subject to constant changes due to tremendous mobility of the larvae, which after becoming isolated from the cyst may either greatly elongate or contract rapidly (Fig. 7). A small invagination becomes observable at the anterior segment within the oral sucker (Fig. 4 and 5). A protractable copulatory organ is situated at the posterior end (Fig. 8).

Despite the slight morphological differences between the two segments of the body, they can be differentiated at first sight.

Optically the light spot of the holdfast organ located in the posterior part of the body separates the two parts (Fig. 6 and 9); beside this, the anterior part contains canals and the reserve bladder'net which are lacking in the posterior part. The two parts differ also in color.

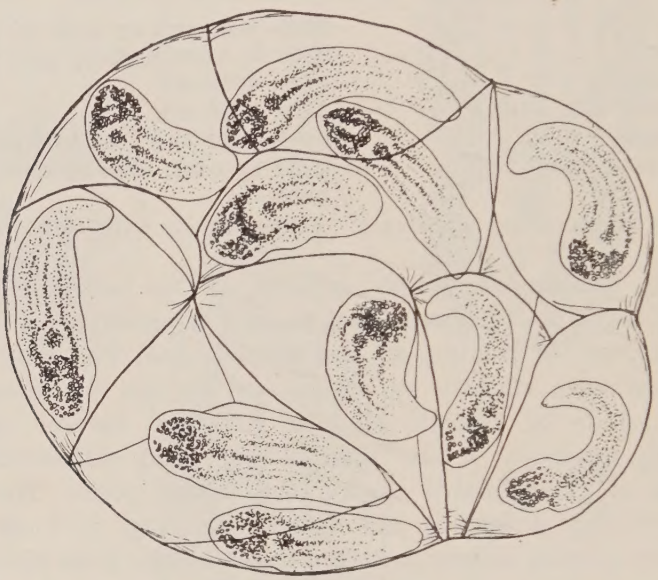

Fig. 9. Metacercariae of *N. pseudattenuatum* (D u b.) — the packet of cysts in the brain of *Scardinius erythrophthalmus* (L.).

The posterior segment is lighter from the moment it becomes filled with large and dark spherical bodies regarded by H u g h e s (1928) as the „dense excretory fluid".

The excretory system of the larvae, similar to the one observed by W i ś n i e w s k i (1958) in *Posthodiplostomulum brevicaudatum* (N o r d m a n n 1832) W i ś n i e w s k i 1958, constins in its anterior part of five longitudinal canals, commissures connecting them, a thick lateral net and a circular canal (Fig. 6, 7). The excretory vesicle and the course of canals in the posterior part of the larval body are difficult to observe. There seem to be four such canals (Fig. 7).

The body of the larvae is at this stage of development quite transparent. It enables to differentiate in live specimens the holdfast organ, sucker, praepharynx, pharynx and esophagus and sometimes even the caeca. Permanent mounts stained with carmin

alum and particularly Blachin lactic acid carmin show the digestive tract much more clearly; it consist of a middle size oral sucker, praepharynx, muscular pharynx, middle long esophagus and caeca. The moderately wide caeca surround the holdfast organ from both sides and terminate posteriorly to it at about the level of the copulatory apparatus. The ventral sucker is weaker and slightly smaller from the oral one. Holdfast organ highly muscular and large, with narrow multilayer band of glandular cells adhering to it posteriorly. Posteriorly to it the differentiated primordia of testes and ovary and a well developed very large copulatory apparatus may be observed. The copulatory apparatus (Fig. 5) may be projected from the body of the larvae (Fig. 8), similarly to Hughes (1927) observations regarding *Neascus ambloplitis*, but this does not occur as frequently as observed by the author in *Posthodiplostomulum cuticola* (Nordmann 1832) Dubois 1937 and *Posthodiplostomulum brevicaudatum* (Nordmann 1832) Wiśniewski 1958. It then resembles a lampshade with conus genitalis projecting from its inside (Fig. 8). The cuticle is already thicker. Specimens stain poorly and with considerable difficulty.

Measurements of encysted live specimens (under cover slip):

body	0.347; 0.665 mm by 0.266; 0.247 mm
anterior segment	0.440; 0.399 mm by 0.276; 0.323 mm
posterior segment	0.260; 0.095 mm by 0.256; 0.247 mm

Measurements of live specimens liberated from the cysts:

body	0.442—0.927 by 0.164—0.295
length of tail	0.175—0.280
oral sucker	0.023—0.030 by 0.023—0.036
ventral sucker	0.017—0.020 by 0.020—0.028
pharynx — length	0.036—0.049
holdfast organ	0.076—0.122 by 0.099—0.140

Measurements of mounted specimens liberated from cysts:

body	0.418—0.722 mm by 0.209—0.380 mm
oral sucker	0.024—0.032 by 0.024—0.036 mm
pharynx (length)	0.024—0.032 mm
ventral sucker (diameter)	0.020—0.028 mm
holdfast organ	0.080—0.100 by 0.100—0.128 mm

The cysts are oval shaped; at this stage they have clear and sharp contours, but are still thin and delicate. They are light and transparent and burst easily. Beside the larval body they also contain a fluid which accounts for their elasticity (Fig. 9). The

larvae move freely and fairly rapidly within the cyst. The cysts form, at least partly out of cells migrating out of the ependyma of the host's brain as a result of his reaction to the parasite and surround the worm with a thin monolayer ring. Originally the nuclei of these cells may be differentiated, but slowly they disappear leaving a thin homogenous capsule enveloping the parasite (Kozicka 1958).

The cysts rarely appear individually. They usually adhere to one another forming larger or smaller packets (Fig. 9). The larvae and cysts of *N. pseudattenuatum* deform, flatten and damage several parts of the brain. The measurements of live cysts observed under a cover slip are usually from 0.528 to 0.792 mm in length and from 0.264 to 0.616 mm in width; they oscillate slightly about this range.

Distribution: Soviet Union: Dvina, Danube, Dnieper and the outlet of Volga; Poland: Lake Drużno, Gołdapiwo, Mamry Północne, Łabędzie, Kortowo, Dgał Duży. Extensiveness and intensity of invasion is very high in case of Lake Drużno, Łabędzie, and Mamry Północne and slight for the remaining lakes (Kozicka 1958).

Intermediate host: *Scardinius erythrophthalmus* (L.) seldom other species of *Cyprinidae* and their fry.

Definitive host: According to Dubois (1938) *Neodiplostomum pseudattenuatum* was found in *Buteo buteo* (L.), *Buteo lagopus* (Pont.) and *Milvus lineatum* (Gray). Sudarikow (1958), who partially followed the development of the parasite, obtained it experimentally in *Milvus korschun* Gmelin and *Corvus cornix* L. This remains in agreement with Dubois assumption that the genus *Neodiplostomum* Raillet occurs in carnivorous birds.

Taxonomy of larva. The anatomical features of the metacercaria *Neodiplostomum pseudattenuatum* correspond to those of a larval species *Neascus* established by Hughes (1927) in the broad sense outlined by Wiśniewski (1958). On this basis *Neodiplostomulum scardinii* Schulman was listed in a previous publication as *Neascus scardinii* (Schulman). Dubois (1938) excluded the larvae of the genus *Posthodiplostomum* and *Neodiplostomum* from the larval genus *Neascus* proposing for them the nomina *Posthodiplostomulum* and *Neodiplostomulum*. Accordingly the metacercaria *Neodiplostomum pseudattenuatum* may be referred to as *Neodiplostomulum pseudattenuatum* (Dubois 1928) Dubois 1932.

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STRESZCZENIE

Autorka podaje opis metacerkarii *Neodiplostomum pseudattenuatum* (Dubois 1928) Dubois 1932 i jej rozwoju, prześledzonego po raz pierwszy w warunkach naturalnych jeziora. Metacerkarie te, dla Polski nowe, znane dotychczas tylko z terenu ZSRR, osiedlają i rozwijają się w mózgu, rzadziej w oczach, głównie u *Scardinius erythrophtalmus* (L.), rzadziej u innych karpiowatych. Incystują się dopiero po pewnym czasie po wniknięciu. Ich patogenne działanie na ryby i ujemny wpływ na liczebność najmłodszego narybku podane zostały w poprzedniej publikacji (Kozicka 1958).